SPECIFICATION

Please enter the following amendments to the specification:

Replace the paragraph at page 19, lines 5-27:

Two different universal 16S rDNA bacterial PCR set of primers and one set of specific oligonucleotides for DNA sequences of the *Candidatus* Endoecteinascidia frumentensis 16S rDNA were used for the amplification experiments. The universal primers were: forward, 8-AG(AG) GTT TGA TC (AC) TGG CTC AG-27 (SEQ ID: 9); reverse 1509-G(GT)T ACC TTG TTA CGA CTT-1494 (SEQ ID: 10) (primer position is according to *E. coli,* Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. 1999. 16S ribosomal DNA amplification for phylogenetic study. J. Bac. 173, 607-703.) and 16SF1 5'-GAG A(G/C)T TTG ATC (A/C/T)TG GCT CAG-3' (SEQ ID:11); 1600R 5'-AAG GAG GTG ATC CAG CC-3' (SEQ ID: 12) (modified from Dorsch, M. & Stackebrandt, E. 1992. Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. J. Microbiol. Methods, 16, 271-279), and the specific ones were EFRU-F1, 5'-CGG TAA CAT AAT AAA TGT TTT TTA CAT TTA TG-3' (SEQ ID:2) and EFRU-R1, 5'-TAT GCT TTT GGG GAT TTG CTA GAT T-3' (SEQ ID:3) (this study). A DNA Engine (MJ Instruments, USA) thermocycler, or a Mastercycler personal (Eppendorf, Germany) was used, cycled as follows: 94°C for 2 min., followed by 30 cycles of 55°C (30secs), 72°C (1 min 15s) and 94°C (15s), and a final elongation step of 72°C for 10 minutes. Total bacterial 16S rDNA from the extracted DNA was amplified from several dilutions to obtain optimal results and a "no-DNA" control was run for each PCR mix. The PCR product was confirmed by 1% agarose gel electrophoresis and ethidium bromide staining.

Replace the paragraph at page 20, line 7 through page 21, line 3:

Putative insert-containing clones (100 clones from each of the larval, zooid and stolon, and 40 from the scallop-control material) were selected from the *E. coli* plates by picking with a sterile toothpick and emulsifying into a 50 pl PCR reaction containing M13/pUC universal primers (forward 5'-GTT TTC CCA GTC ACG AC-3' (SEQ ID:13); reverse 5'-CAG GAA ACA GCT ATG AC-3' (SEQ ID:14)) and patched onto LB agar containing ampicillin, X-gal and IPTG (as above) for subsequent plasmid isolation. PCR reactions were cycled as follows: 94°C for 5 minutes, followed by 30 cycles of 50°C (30secs), 72°C (1min 30s), 94°C (15secs), with a final elongation step of 72°C for 10 minutes. Inserts of the appropriate size (approximately 1700 base pairs) were confirmed by 1% agarose gel electrophoresis. Positive PCR products were ethanol precipitated and resuspended in sterile distilled H_2O. Restriction fragment length polymorphism (RFLP) analysis was carried out using the restriction enzymes *HaeIII* and *HhaI* (Promega, USA). The restricted PCR products were electrophoresed through a lx Tris- acetate (40mM), EDTA (1mM), 3% wide-range agarose gel (Sigma, USA) and stained with ethidium bromide. Kodak 1-D system and software (Kodak, USA) was used to capture the resultant RFLP patterns. Clones representative of each different pattern observed were isolated and grown up in LB broth with ampicillin (75 pg/ml) overnight and stored frozen with 10 % glycerol at -80 °C. Plasmid DNA was isolated from representative clones for DNA sequence analysis according to the manufacturers instructions (Qiagen Spin Mini-Preps, UK). DNA sequencing was performed using the M13 universal primers and ABI BigDye chemistry (PE Applied Biosystems, USA) and analysed on an ABI 377 DNA sequencer (PE Applied Biosystems, USA).

Replace the paragraph at page 21, lines 7-14:

The first approach to the analysis of the microorganisms associated to *Ecteinascidia turbinata* was done using total DNA isolated from adult zooids. Direct sequencing of the PCR amplification fragment obtained with this DNA and "universal" eubacterial primers 16SF1 (5'-GAGA(G/C)TTTGATC(A/C/T)TGGCTCAG-3' (SEQ ID:11)) and 1600R (5'-AAGGAGGTGATCCAGCC-3' (SEQ ID:12)) (modified from Dorsch & Stackebrandt, 1992) resulted in a clean sequence, indicating the prevalence of this microorganism in the tunicate. It is defined above as SEQ ID 1.

Replace the paragraph at page 21, line 16 through page 22, line 5:

With the aim to confirm this result, specific oligonucleotides were designed for the *Candidatus* Endoecteinascidia frumentensis 16S rDNA. These oligonucleotides (EFRU-F1, 5'-CGG TAA CAT AAT A AA TGT TTT TTA CAT TTA TG-3' (SEQ ID:2) and EFRU-R1, 5'-TAT GCT TTT GGG GAT TTG CTA GAT T-3' (SEQ ID:3)) were used as primers for the PCR amplification experiments performed with total DNA isolated from adult zooids from different locations around the world (Formentera, Menorca, Túnez, Cádiz, Cuba, Florida, Puerto Rico) and with DNA obtained at different phases of development (stolon, embryos, larvae, buds and adult zooids). An amplification band with the expected size was obtained in all the cases, and RFLP and sequence analysis showed that *Candidatus* Endoecteinascidia frumentensis was present in all those samples. As a control, total DNA from a closely related organism, *Ecteinascidia conklini* was isolated, and PCR experiments were performed. No amplification was obtained with the *Candidatus* Endoecteinascidia frumentensis specific primers, although bands of the right size could be seen when universal eubacterial primers were used (data not shown). Positive results were also obtained when *E.*

turbinata DNA was used (both with a sample collected at the same area as *E. conklini* was and with a sample from a different location), with universal and specific primers.

Replace the paragraph at page 27, line 28 through page 28, line 23:

Initial in situ hybridisation studies were carried out using a biotinylated universal bacterial 16S rRNA probe to identify sites of potential interest (EUB338 5'-GCT GCC TCC CGT AGG AGT-3' (SEQ ID:7), and a control probe NON-EUB338 5'-ACT CCT ACG GGA GGC AGC-3' (SEQ ID:8)) (Amann, R.I. Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R. & Stahl, D.A. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analysing mixed microbial populations. Appl. Environ. Microbiol. 56, 1919-1925.). Other controls consisted of incubations with no probe, and panels of attached bacterial cells (*E. coli*) which had been fixed and processed as the sections. Hybridisation was carried out at 45°C for 3 hours in buffer (0.9M NaCl, 20 mM Tris-HCl pH7. 2, 1 x Denhardts, 0.1% SDS, 5mM EDTA, 0. 1 mg/ml Poly (A)) with 2.5 ng/μL of probe which had been reconstituted in TE. Hybridisation chambers with dual ports were used to prevent evaporation (Grace BioLabs: 22mm x 22mm chamber for each panel of sections on the slide). Following hybridisation the slides were washed 2 X 15 minutes in wash buffer (0.9M NaCl, 20mM Tris-HCl pH 7.2, 0.1% SDS) at 48°C. Binding of probe was visualised using Avidin-DN (Vector Labs) as recommended by the manufacturer. Sections were mounted in Vectorshield (Vector Labs) and viewed on a Zeiss Axioskop with fluorescent attachments. For visualisation with alkaline phosphatase (AP) the slides were washed after hybridisation and incubated in AP conjugated anti-biotin antibody (Vector Labs) diluted 1: 1000 in buffer (20mM sodium phosphate, 0.9% NaCl, 0.1% Tween, 0.1% BSA) at 4°C overnight. They were then washed and visualised using a BCIP/NBT-AP substrate kit (Vector Labs).